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Remediation Mechanism of Microbial Corrosion for Iron Artifacts Buried in Soil by Using Allium Sativum (Garlic Extract) as a Natural Biocide

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Abstract

The purpose of this study is to examine the bio-corrosion of an ancient knife that was discovered in burial soil and to assess the effectiveness of garlic extract (GAE) as a biocide. The knife was swabbed for microorganisms, and those organisms were subsequently cultured in tryptic soy broth at 37 degrees Celsius for 24 hours. The knife was analyzed using scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS), the corrosion products were analyzed using X-ray diffraction, and the garlic extract was analyzed using gas chromatography-mass spectrometry (GCMS) and Fourier transform infrared spectroscopy (FT-IR). Coupons constructed of synthetic materials with the same chemical composition as the knife under study were manufactured and then submitted to isolated species of bacteria to examine the behavior of microbial corrosion. Scanning electron microscopy was used to examine the biofilm that had formed on the coupons' surfaces. Results indicated that the knife's blade seemed to be made of carbon steel and that five distinct species of bacteria and six different types of fungus were found. The bacteria were determined to consist of four distinct species of iron-oxidizing bacteria (IOB) and one kind of acid-producing bacteria (APB). Sessile IOB was quantified in terms of CFU to examine the bacterial proliferation on the agar plate's surface. A compound in GAE that is rich in sulfur and may block the development of bacteria was discovered using GCMS. The active ingredient in garlic responsible for its antibacterial properties against both Gram-positive and Gram-negative bacteria is allicin. Therefore, GAE may be offered as a natural biocide to replace more hazardous commercial biocides.

Keywords: Allium Sativum; Bio-corrosion; Garlic Extract; Iron Oxidizing Bacteria; Eco-Friendly Biocide.

1. Introduction

Microbiologically induced corrosion, often known as MIC, is an electrochemical process in which microorganisms speed up the metal's deterioration [1]. Because of their capability to attach to metallic surfaces and produce biofilms, they can impact corrosion by modifying the electrochemical conditions at the metal/solution interface [2]. Near metal surfaces, some strains of microorganisms release metabolic products that are very corrosive to the metal [3]. The formation of biofilms is dependent on the bacteria being in a sessile state [4]. They can utilize extracellular electrons or generate highly concentrated corrosive metabolites underneath the biofilms]; sessile cells that are attached to the steel surface are directly responsible for MIC [5].

Sulfate-reducing bacteria (SRB) and thiosulfate-reducing bacteria (TRB) are the primary microorganisms responsible for the biocorrosion process in anaerobic settings [6]. Recent years have seen a proliferation of research on the minimum inhibitory concentration (MIC) in the presence of lithotrophic Fe-oxidizing bacteria (FeOB) and heterotrophic bacteria, particularly Bacillus and Pseudomonas strains [7]. The Fe-oxidizing bacteria are autotrophic and have a wide range of structural diversity [8]. However, they all use oxygen to oxidize ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}), which results in

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the formation of insoluble precipitates of oxides ($Fe_2O_3.H_2O$) or iron hydroxides ($Fe(OH)_3$) in metal substrates [9]. These precipitates appear as brownish or orange tubers [10].

This kind of bacterial biocorrosion is often brought on by a specific heterogenic group, which consists of the genera Gallionella, Sphaerotillus, Crenothrix, and Leptothrix [11]. Fungi that grow on iron artifacts may potentially cause harm to the artifacts because of their porous nature [12]. Their development on metal surfaces is dependent on metabolites, which provide them the ability to adapt to different situations for both the environment and the nutrients [13]. Even though data on the fungal capacity to adapt to metal surfaces is limited, most fungi that grow on metal surfaces engage in the corrosion process, which involves it in the process of functional development and metabolism. This is the case even though data on this topic is limited [14]. The first indicators that fungus has impacted metal surfaces and is exploiting them to meet its nutritional demands are changes in the color of the metal, which is one of the early symptoms of corrosion [15].

To avoid or reduce the negative effects of microbial activity on metals and alloys like carbon steel, it is necessary to have methods for the prevention and control of MIC [16]. The use of biocides in chemical treatments is a means of preventing the formation of biofilms [17]. When choosing biocides, it is necessary to consider several factors, including the antimicrobial activity range, the cost, and the influence on the environment [18]. Formaldehyde, ethylene glycol, glutaraldehyde, sodium molybdate, and quaternary ammonium salts are only some of the chemical biocides that have been shown to successfully inhibit microbial activity and the growth of biofilm [19]. On the other hand, environmental regulations concerning the use of these chemical biocides provide a persistent obstacle for the sector [20]. Compounds derived from plants, for instance, have lately attracted more interest as potential natural biocides [21]. As a result of their low cost and little impact on the environment, plant derivatives are becoming more popular as a means of MIC control [22]. Natural biocides are used on both organic and inorganic materials since it is believed that they pose less of a risk to human health and are friendlier to the natural environment [23].
A good number of these chemicals may be obtained from plants, and they can be used in their natural condition, as crude extracts, or as essential oils [24]. The prevention of bio-corrosion has been accomplished with the use of eco-friendly biocides such as plant extracts and antimicrobial peptides [25]. Many plant derivatives have been utilized to prevent chemical corrosion, including Ervatamia Coronaria, Phyllanthus Amarus, Garlic peel extract, Aloe vera, Lemon verbena, Gossypium Hirsutum, Bamboo leaves, Artemisia Pallens, orange peel, Musa Paradisica, and Tagetes erecta [26]. Garlic (Allium sativum) is the oldest vegetable that has been utilized in medicine owing to the broad biological activity that it has, which includes antibacterial, anticancer, and antioxidant qualities, among others [27].

In addition, garlic oil and extract made from garlic peel have been used as natural corrosion inhibitors for carbon steel in situations that are acidic [28]. Garlic has several benefits that set it apart from other plant extracts, including the fact that it is readily available and very affordable [29]. It consists of a mixture of organosulfur compounds that have shown conclusive evidence of possessing antibacterial properties [30]. Several researchers have investigated the effectiveness of garlic extract as a corrosion inhibitor, intending to reduce acid corrosion [31]. These analyses revealed the presence of sulfur-containing compounds, which are crucial to corrosion-inhibiting activity [32]. The purpose of this study is to explore the bio-corrosion of an archaeological knife that was excavated from burial soil and to evaluate the effectiveness of garlic extract as a natural biocide under these circumstances [33].

2. Material and methods

2.1. Sampling

For this investigation, a knife made of iron and high-carbon steel that is housed at a national museum was chosen. It is observed that the microbiological infection was caused by the burial soil, because of the uncontrolled storage process, or both together. Accordingly, two microbiological swabs (S1, S2) and two samples from corrosion powder (C1, C2) were taken from the knife according to the condition in which it was found. These swabs were kept so that the kinds of bacteria and fungus that were present, as well as the influence those bacteria and fungi had on the corrosion processes of the investigated knife, could be determined. In addition, a tiny sample was removed from the knife to carry out the metallographic investigation [34].

This sample included both the metallic structure and the exterior corrosion layer that was present on the knife. After taking a sample off the edge of the knife and mounting it as a cross-section using Araldite glue or another embedding process, the samples were polished using silicon carbide sheets with grits of 400, 600, 800, and 1200 respectively. After being polished, the samples were degreased with

ethanol and then rinsed with distilled water. To investigate the microbial corrosion behavior of an artifact, coupons made of high-carbon steel with the same chemical composition as the item under research were manufactured and subjected to isolated species of bacteria. A guillotine was used to chop the coupons down to the dimensions of 20 mm by 50 mm by 1 mm [35].

2.2. The examination and analysis of the studied object

The cross-section sample that was extracted from the knife was examined with a polarized light microscope and analyzed using a scanning electron microscope combined with energy dispersive spectrometry (SEM-EDS) to demonstrate the chemical composition and characteristics of the microstructure. The scanning electron microscope used by the JEOL Company was a model JSM-5410, and it had an energy-dispersive spectrometry (EDS), INCAPentaFETx3 spectrometer with a 20 kV power source. The surface of the knife was analyzed using a USB Digital Microscope so that the different stages of corrosion could be distinguished and studied following their respective colors [36].

2.3. Determination of colony-forming units (CFU) of bacteria

Aseptically putting each sample of microbiological isolations into a medium consisting of tryptic soya broth (Oxoid®) and incubating it at 37 degrees Celsius for 24 hours was done so that colony-forming units could be determined. After preparing serial dilutions, 0.1 milliliters of each dilution were spread out on plates constructed of tryptic soya agar (brand name: Oxoid®). Plates were kept in an incubator at 37 degrees Celsius for a whole day. Plates were only analyzed in terms of their colony count if they contained between 30 and 300 colonies [37]. The number of CFU that were present in each ml of sample was then determined by using the following equation (1):

CFU/ml = no. of colonies x 1/ dilution (1)

2.4. Isolation of tested micro-organisms

2.4.1. Isolation of bacteria:

Macconkey (Oxoid®) and Tryptic soya agar plates were then streaked with samples of the cultured broth that had been incubated. The plates were kept in an incubator at 37 degrees Celsius for a whole day. After being cleaned, the colonies were picked up and cultivated on tryptic soy broth with 70 percent glycerol so that they could be identified in greater detail [38].

2.4.2. Isolation of fungi:

The dilution plate approach was the one that was used to carry it out [39]. Following the addition of one milliliter of aliquots to a sterile Petri plate, twenty milliliters of molten rose Bengal chloramphenicol agar (RBCA) medium were poured over the top [40]. Incubate the plates at a temperature of 28 degrees Celsius for a week. The resulting isolates were analyzed for their macroscopic and microscopic properties to determine their identities, and they were then maintained on PDA slants [41].

2.5. Identification of the bacterial isolates

2.5.1. Microscopically examination:

Gram stain was applied to films made from pure suspicious cultures, and then those films were studied using a microscope.

2.5.2. Biochemical identification

2.5.2.1. Catalase activity test

A sterile loop was used to pick up the purified suspicious colonies, and then those colonies were put onto the surface of a glass slide. To be more specific, one or two drops of hydrogen peroxide solution with a concentration of 3% were added, and then the cover slide was attached. The quick formation of gas bubbles was seen as a favorable response [42].

2.5.2.2. Oxidase test

The pure culture was streaked onto filter paper that had been wet with an oxidase reagent before the oxidase test was carried out. If the hue changes to a mauve, violet, or deep purple within ten seconds, the test is positive.

2.5.2.3. Indole test

After incubating the culture at 37 degrees Celsius for 48 hours in 1% peptone water, 1 milliliter of ethyl ether was added to the mixture. After violently shaking the tubes, we let them stand for a period until the ether rose to the top. In each tube, 0.5 milliliters of Kovac's reagent were added by slowly pouring it down the tube's side. After 10 minutes, a positive reaction was determined to have occurred when a red ring appeared on the surface of the solution [43].

2.5.2.4. Methyl Red Test

After adding pure culture to a tube containing five milliliters of buffered glucose broth, the tube was then heated to 37 degrees Celsius for one day [44]. Five drops of the Methyl Red reagent were put into each tube. The appearance of a red hue during the test was taken to indicate a successful result.

2.5.2.5. Voges-Praskauer test

For 48 hours culture was incubated at 37°C in 5 ml buffered glucose phosphate broth, 1 ml was taken in a test tube and 0.6 ml of alcoholic solution of alpha–naphthol and 0.2 ml of 4% potassium

hydroxide solution were added. The tubes stood for 24 hours and the pink coloration of the mixture was recorded as a positive test [45].

2.5.2.6. Citrate utilization test

Pure cultures were used to pierce slants and butts in Simmon citrate agar tubes, which were then placed in an incubator at 37 degrees Celsius for 48 hours. The appearance of a blue coloring was indicative of the use of citrate [46].

2.5.2.7. Urease test

The Christensen medium was seeded with the possible isolates, and the dish was then heated to 37 degrees Celsius for a whole day. The appearance of a pink tint indicated that urea had been hydrolyzed. After a further 24 hours of incubation, the negative tubes were subjected to the second round of testing [47].

2.5.2.8. Nitrate reduction test

The culture that was going to be examined was inoculated into 5 ml of peptone broth that contained 0.1% potassium nitrate, and it was incubated at 37 degrees Celsius for four days. After that, 1 ml of a solution that contained 8 grams of sulphanilic acid in 100 ml of 5 N acetic acid was added and mixed, and then a solution that contained 5 grams of alpha–naphthylamine in 100 ml. The formation of a crimson hue served as an indication that the test was positive [48].

2.5.3. Detection of Ornithine decarboxylase (ODC)

Just below the surface of the medium containing ornithine decarboxylase, suspected colonies were infected with the bacteria. After adding one milliliter of sterile mineral oil to the top of the medium, it was then heated to 37 degrees Celsius for twenty-four hours. A positive oxygen demand concentration was shown by turbidity and a violet tint after incubation [49].

2.5.4. Detection of L- lysine decarboxylase (LDC)

Just below the surface of the L-lysine decarboxylase media, suspected colonies were injected with the appropriate bacteria. After adding one milliliter of sterile mineral oil to the top of the medium, it was then heated to 37 degrees Celsius for twenty-four hours. After incubation, a positive LDC is indicated by turbidity as well as a hue that is violet [50].

2.5.5. Detection of Arginine decarboxylase (ADH)

Just below the surface of the arginine decarboxylase media, suspected colonies were injected with the appropriate bacteria. After adding one milliliter of sterile mineral oil to the top of the medium, it was then heated to 37 degrees Celsius for twenty-four hours. After incubation, a positive ADH is indicated by turbidity as well as a hue that is violet [51].

2.5.6. Fermentation of sugars

Five milliliters of peptone water containing one percent bromocresol purple indicator were given one percent of each of the following sugars (lactose, sucrose, maltose, mannose, glucose, mannitol, and xylose). To collect a gas, Durham's tubes were turned inside out and placed inside the test tubes. Following incubation at 37 degrees Celsius for seven consecutive days, the reactivity of the infected tubes was observed daily. The appearance of the hue pink suggests a successful outcome [52].

2.6. An experimental study of the effect of isolated bacteria on the artificial carbon steel coupons

The IOB was grown on the artificial carbon steel coupons inside glass tubes in the presence of an ammonia ferric citrate medium (CFA). This medium was comprised of 0.5 g/l $(NH_4)_2SO_4$, 0.2 g/l $CaCl_2.6H_2O$, 0.5 g/l $MgSO_4.7H_2O$, 0.5g/l $NaNO_3$ and 10.0 g/l Ammonia ferric citrate and 0.5 g/l K_2HPO_4. The experiment was conducted using bottles with a capacity of 100 mL, each of which contained 80 mL of CFA culture media. At first, a monitoring experiment was carried out to investigate the microbial growth in culture media over 35 days. The color of the media changed from green to a reddish-rust hue, which indicated the oxidation of the iron that was present in the broth medium. This color change allowed the positive iron precipitating bacteria to be identified visually [53].

2.6.1. After the exposure of iron coupons to corrosive bacteria

X-ray diffraction analysis was used to investigate the corrosion products that had developed on the surface of the coupons. A Philips X-Ray, diffract meter type: pw1840 with Cu k& Radiation was used to analyze the two samples that were obtained to represent the corrosion products that were found on the surface of the iron coupons. An SEM analysis was carried out on the specimens that had been exposed to the control medium for 35 days, as well as on the specimens that had been introduced into the media containing biocides for the same amount of time. After being cleaned thoroughly with distilled water to get rid of any free cells, the coupons were dried using a heater set to 37 degrees Celsius for one hour. Using a scanning electron microscope (JEOL model JSM G510 LV) with a high vacuum mode, an

SEI detector, and 20 kV of power, the samples were examined [54].

2.7. Preparation of garlic extract

In the Egyptian city of Qena, garlic bulbs were purchased at a neighbourhood market. After removing the cloves from fresh garlic bulbs and peeling them, the bulbs were minced and pressed until some garlic juice was collected. No other ingredients or water was used in this process. The garlic extract was filtered immediately using a muslin cloth, and the filtrate was 100% aqueous garlic juice. This juice was used in tests for its antibacterial and antifungal properties on the same day [55].

2.8. Chemical analysis of garlic extract

2.8.1. Gas chromatography analysis

To do gas chromatography (GC) and GC-mass spectrometry (MS) investigations of sulfur compounds, a sample of the biocide was extracted with ethyl acetate, and then the ethyl acetate extracts were injected. Both techniques are used to analyze sulfur compounds. GC was carried out on a Shimadzu GC-17A gas chromatograph that was outfitted with a Hewlett-Packard-WAX (i.d. 0.32 mm 3 30 m) column; the column temperature was held at 60°C for 3 minutes, increased to 4°C/min to 200°C and held for 10 minutes; the carrier gas was He (53 kPa, split ratio 1/3); and detection was carried out using flame photometry. The GC-MS analysis was carried out using a JEOL GC-mate under the same conditions except for the flow rate of He (which was reduced to 1.2 mL/min) and the method of detection, which was electron impact mass spectrometry (TIC model) [56].

2.8.2. Fourier transform infrared spectroscopy (FT-IR)

FT-IR analysis was used to determine the identities of the functional groups that were present in the garlic extract. Following the drying of a few drops of garlic extract on a glass plate for FT-IR analysis, the dried sample was crushed with the addition of potassium bromide in a ratio of 1:100, and the pellet was then fixed in the sample container and examined using FT-IR (model Jasco) in the mid IR range 400-4000 cm^{-1} [57].

2.9. Antibacterial properties of garlic extract

2.9.1. Agar well diffusion assay for bacteria

The antibacterial activity was examined using a technique known as well diffusion. Bacterial growths that had been allowed to develop overnight were used to swab the surface of Tryptic Soy Agar (TSA) plates. The top of the agar was sterilely drilled into wells with a diameter of 6 millimeters, and each well contained 50 microliters of the respective extract. After an incubation period of 24 hours at 37 degrees Celsius, the plates were allowed to rest at room temperature for 15 minutes before the pre-diffusion step. In the inhibitory zone, which was measured with a ruler in millimeters, the antibacterial activity of the extract was seen [58].

2.9.2. Determination of the minimum inhibitory concentration (MIC) of garlic extract

After overnight incubation, the bacteria that had been isolated were subcultured in tryptic soy broth (TSB). In each well of a 96-well plate, we added varying concentrations of the garlic juice's source along with bacterial growth samples that were each 100 microliters in volume. The minimum inhibitory concentration (MIC) was determined during an incubation period of 24 hours at a temperature of 37 degrees Celsius. After adding 40 l of p-iodonitrotetrazolium violet (INT) (0.2 mg/mL, Sigma-Aldrich) to each well of the microplate and reincubating it at 37 degrees Celsius for 30 minutes, the results showed that bacterial growth was inhibited and there was no evidence of metabolic activity [59].

In the INT test, the minimal inhibitory concentration, or MIC, was determined as the lowest concentration that prevented a color change, as was explained previously. The bactericidal effect was defined as a 99.9% drop in CFU (3 logs) in the initial inoculum over a 24-hour incubation period, and the Minimum Bactericidal Concentration (MBC) testing was also calculated. The MBC was calculated by moving 50 l from each well of an overnight MIC plate (and/or higher) to sterile (TSA) new plates and then re-measuring the concentration of the bacteria. After 24 hours at 37 degrees Celsius, the number of viable colonies was determined. This test has a detection threshold of 101 CFU/mL as its minimum required concentration [60].

2.10. Antifungal efficiency of garlic extract

The agar well diffusion technique was used to investigate the antifungal characteristics of the sample. A new fungal culture was pipetted onto the middle of a clean Petri plate and allowed to sit there for one milliliter. Following the completion of the chilling process, molten Potato dextrose agar (PDA) was added to the Petri plate containing the inoculum, and the contents were well mixed. Following the completion of the solidification process, sterile cork borers were used to drill wells into the agar plates that contained the inoculums (6 mm in diameter) [61].

After that, one hundred microliters of the extract were poured into each of the relevant wells. After chilling the plates for thirty minutes, the extracts were allowed to completely diffuse throughout the agar. After that, the plates were kept in an incubator at 25 degrees Celsius for between 48 and 72 hours. After incubation, the diameter of the clear zone of

inhibition surrounding the discs was measured in millimeters to determine the results (mm) [62].

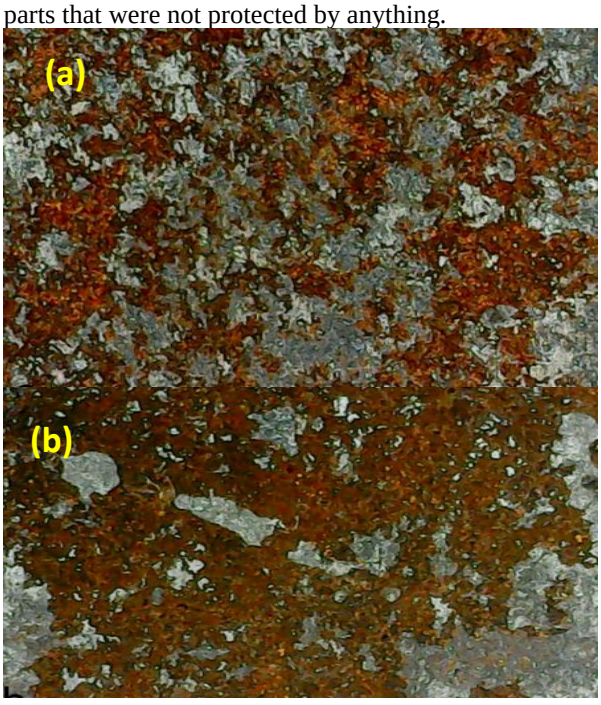
3. Results and discussion

3.1. Description, examination, and analysis

The archaeological iron knife that was recovered from the burial ground and is now housed in the national museum served as the subject of this investigation. The blade is 367 millimeters long, and its widest point is 10 centimeters. It was coated with a coating of corrosion products that were black, red, and brown (Fig. 1).

Fig. 1: Shows the knife before the treatment

Examining the surface of the knife using a USB Digital Microscope was done. The USB had blisters with a glossy appearance, which were most likely caused by a corrosion phenomenon known as weeping. Archaeological conservators refer to the appearance of "tears" or "sweat" on the surface of unearthed iron as "weeping," and they use this term to describe the phenomenon [63]. Weeping droplets of $FeCl_3$ were mentioned in the previous sentence see Fig. 2. Under the USB Microscope, two shades of brown corrosion were observed: dark brown and light brown. The original cooler of the knife was seen under a USB microscope as well, even though it was coated with corrosion products and looked grey in parts that were not protected by anything.



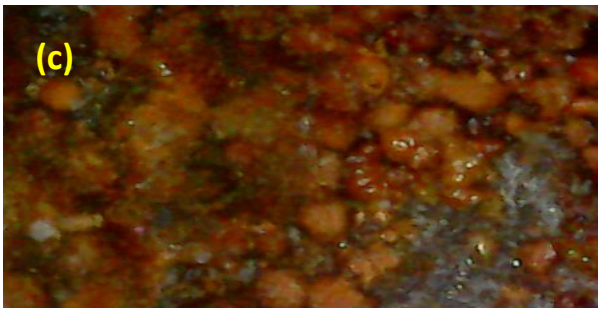
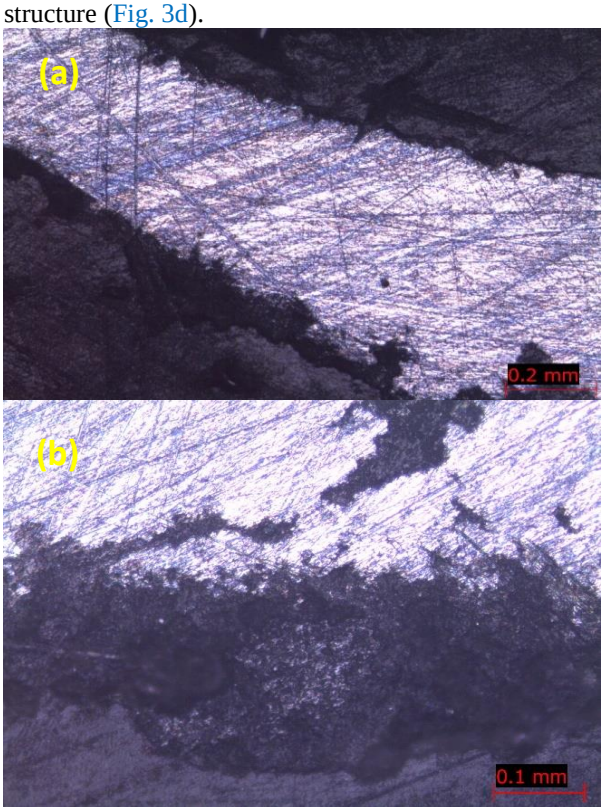


Fig. 2: Shows a USB Digital Microscope of the iron knife at three different magnifications (a) 10 X (b) 20 X and (c) 100 X

Metallographic analysis of the sample of the knife was done so that it could demonstrate the microstructure of the metal as well as characteristics of the degradation that were spread over the metal's surface. Regrettably, to retrieve this information, one must often do intrusive operations on the artifact. The analysis revealed that the knife was in excellent preservation condition; a thin coating had developed on the original surface of the knife, and the metallic structure underlying it was still in fine shape, as seen in the image (Fig. 3a, b). As can be seen, the corrosion layer looked to be rather thick in several locations and included relatively few remnants of the original metal structure (Fig. 3d).



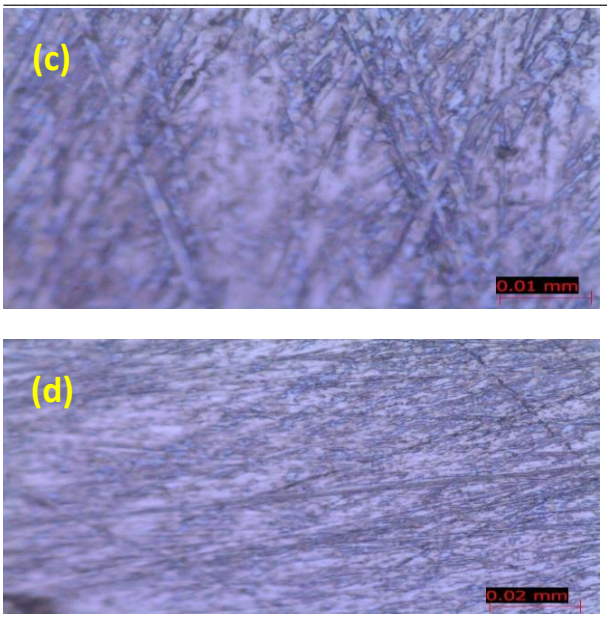
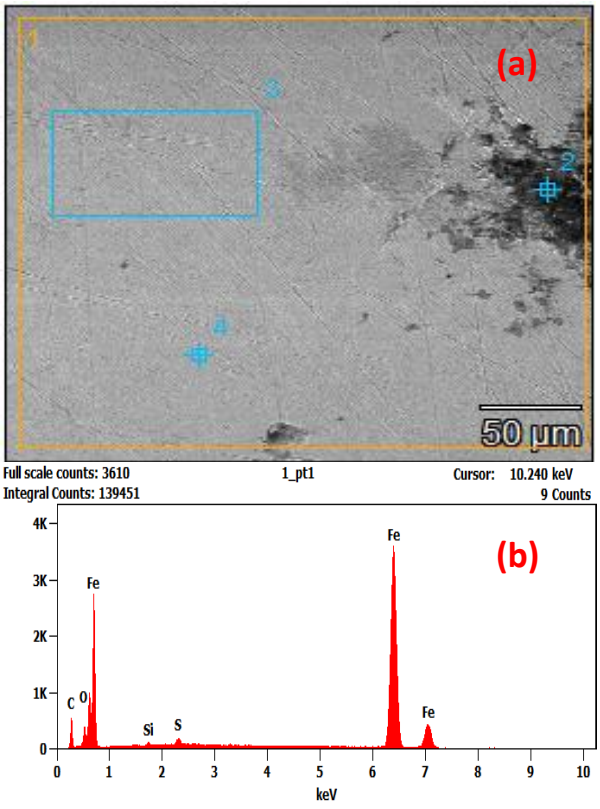


Fig 3: Representative the images of polarizing light microscopy for the cross-section of the coupon at different magnifications (a) 0.2 mm/cm, (b) 0.1 mm/cm, (c) 0.01 mm/cm, and (d) 0.02 mm/cm.

Cracks running in a longitudinal direction were also seen in the metal framework. Based on the findings of the SEM-EDS analysis (Figs. 4, 5), it was determined that the knife was made of high carbon steel in the proportions shown in Table 1.



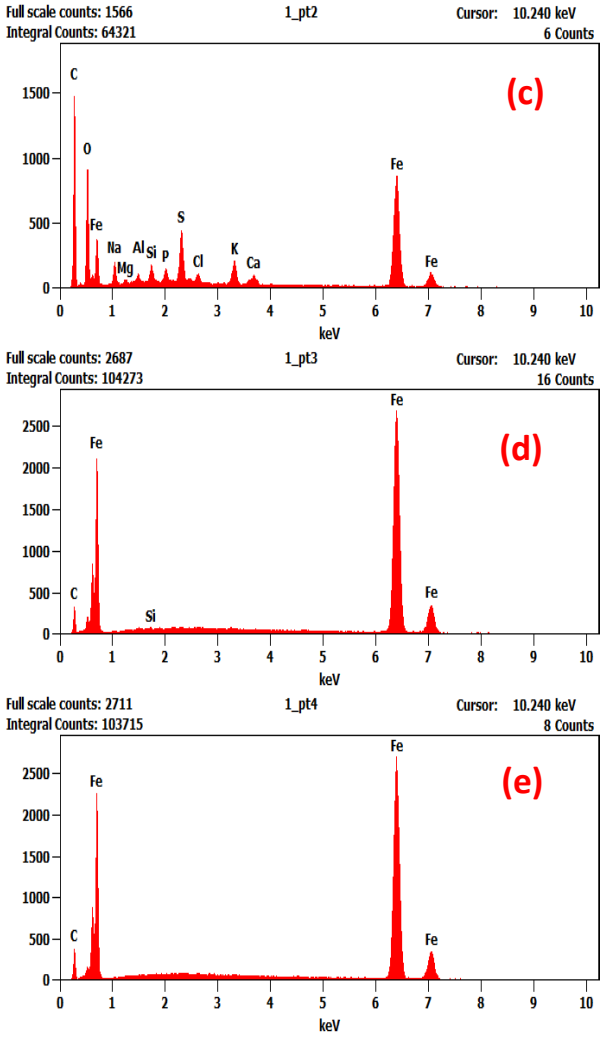
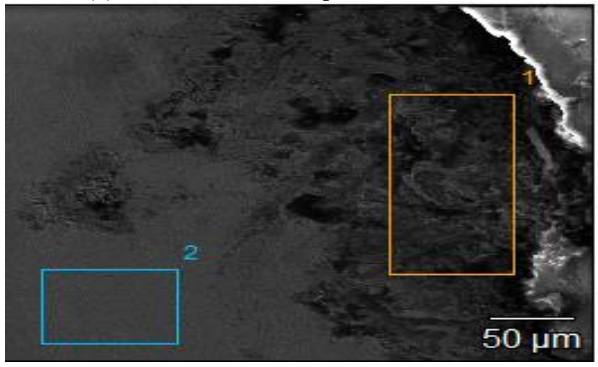


Fig. 4: Representative the SEM&EDS of four points of analysis for the knife (a) SEM Image, (b) EDS of spot 1, (c) EDS of spot 2, (d) EDS of spot 3, and (e) EDS of spot 4 and you can observe the point 2 chart (c) with the corrosion products.



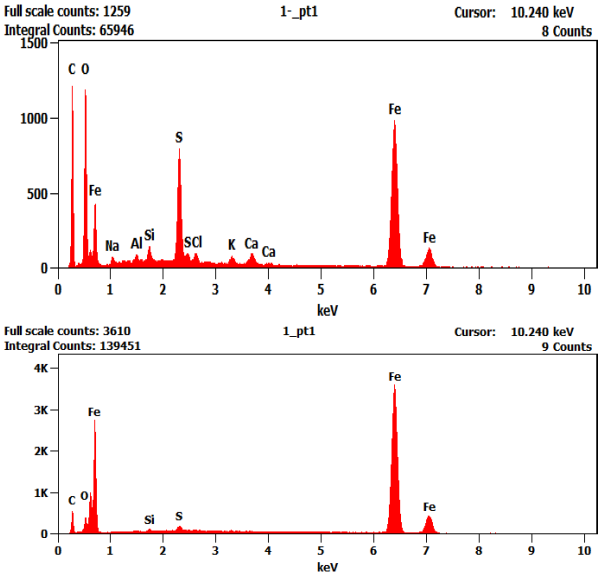


Fig. 5: Representative the SEM&EDS of the (a) corroded point and (b) none corroded point for the knife

3.2. Incidence of bacteria among samples.

Sample (S1) exhibited the greatest prevalence among all the isolated samples with 68 cfu of 105, followed by sample (C1) with 32 cfu of 105. All the other samples had a prevalence of less than 50 cfu of 105. The second sample (C2) has 38 cfu out of 104. Finally, the results of the sample (S2) showed the lowest prevalence, with 29 cfu out of 103, as shown in Table 1. Table 2 was designed with the results of identification by biochemical tests, and it revealed that the most prevalent organism was Gallionella Ferruginea with a percentage of 37.5% (6/16).

This was followed by Chlorochromatium Aggregatum and Thiobacillus Ferrooxidans, each of which had a percentage of 18.75% (3/16). There was a 12.5% chance that Leptothrix Ochracea was isolated from the sample (2/16). Both Sphaerotililus species and Arthrobacter (Siderocapsa) Castelli were found to have a percentage of 6.25% (1/16), which was the lowest reported value for each of them [64].

3.3. Identification of isolated bacteria and Fungi

We identified 5 species of bacteria with biochemical tests as shown in Table 3

Table 1: shows SEM&EDS and CS analysis results of the knife

Samples	Spots	Fe	C	Si	Al	Na	P	S	K	Ca	O	Mg	Cl	Total, %
Sample 1	Spot 1	90.61	6.77	0.31	-	-	-	0.79	-	-	1.52	-	-	100
	Spot 2	37.83	27.60	1.20	0.60	2.74	0.91	3.61	2.11	0.96	21.61	0.18	0.65	100
	Spot 3	94.27	5.67	0.70	-	-	-	-	-	-	-	-	-	100
	Spot 4	93.58	6.42	-	-	-	-	-	-	-	-	-	-	100
Sample 2	Spot 1	42	22.90	0.80	0.41	0.94	-	6.74	0.94	1.02	23.98	-	0.70	100
	Spot 2	93.95	6.05	-	-	-	-	-	-	-	-	-	-	100
Sample 3 (SCA)		95.4	4.6	-	-	-	-	-	-	-	-	-	-	100

Table 2: Prevalence of bacteria isolated from samples

Samples	Colony Forming Unit (cfu/ml)
Sample (S1)	68 $x10^5$
Sample (S2)	29 $x10^3$
Sample (C1)	32 $x10^5$
Sample (C2)	38 $x10^4$

Table 3: Biochemical tests for identification of isolated bacteria

Biochemical tests	G. Ferruginea	L. Ochracea (Sphaerotililus Spp.)	T. Ferrooxidans	C. Aggregatum	A. Castelli
Gram staining	Gm –ve	Gm –ve	Gm –ve	Gm –ve	Gm +ve
Morphology	Curved bacteria	Filamentous bacteria	Rods	Rods	Short irregular rods
Oxidase	+	+	+	+	-
Catalase	-	-	-	-	+
Indole	+	-	-	-	-
Methyl red	-	-	+	-	V
Voges Proskuaer	+	+	-	+	V
Citrate utilization	+	-	+	-	V
Urease	-	+	+	-	V
Nitrate reduction	-	-	-	+	-
ODC	+	-	-	+	V
LDC	-	-	-	+	-
Arginine Di hydrolase	+	+	-	-	-
Sugar fermentation					
Lactose	+	+	+	-	+

Sucrose	+	V	+	+	+
Maltose	+	+	+	-	+
Mannose	-	V	-	-	V
Glucose	+	+	+	V	-
Mannitol	+	+	V	-	-
Xylose	-	-	-	V	+

(+): positive; (-): negative; V: variable

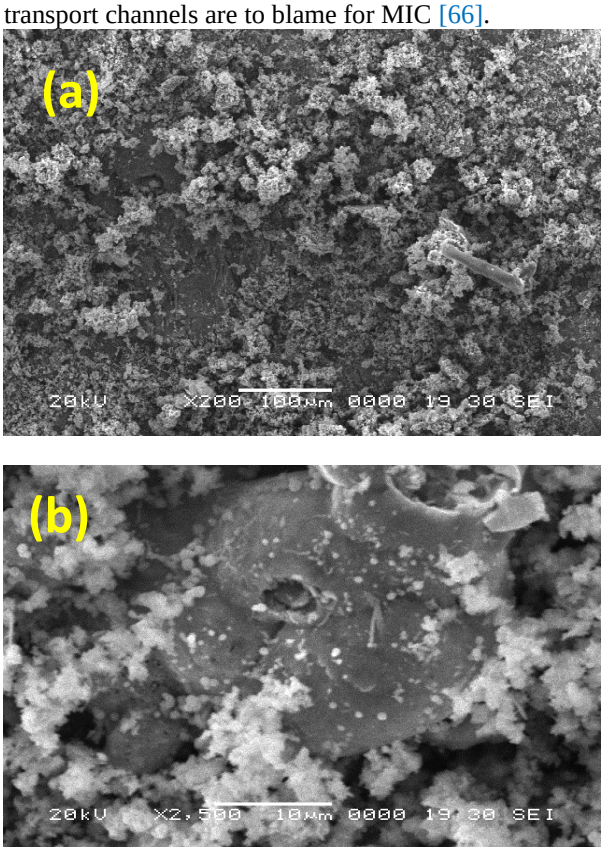
The use of a stereomicroscope allowed for the identification of the isolated fungi, which turned out to be six different species belonging to four different genera. These fungi were named as follows: Aspergillus Flavus, Aspergillus fumigatus, Aspergillus Niger, Aspergillus Sydowii, Rhizopus sp., and Ulocladium botrytis.

3.4. Studying the artificial coupons exposed to the isolated bacteria species

The micrographs were carried out after removing the coupons from the enrichment of microbial consortia on the metallic surface as seen in Fig 6.

Fig. 6: Shows the iron coupons during corrosion in bottles and the coupons after the test

The coupons were acquired after an experiment lasting 35 days in which a specimen was submerged in a medium that included the examined separated bacteria. During the whole process of biofilm development, the bacteria displayed microbial adherence. Fig. 7 is a micrograph that reveals rods and cocci in unevenly dispersed cell agglomerates on the surface of the carbon steel. These agglomerates may be seen on the surface of the steel. It was also discovered that there were some flakes, which were most likely caused by the extracellular products (EPS) of the metabolic activity of the IOB. EPS has the potential to affect the physicochemical environment in a way that makes it more favorable for the electrochemical processes that are responsible for localized corrosion. This may lead to the production of biofilms on metal surfaces that are non-homogeneous and porous [65]. It is well known that biofilm may grow on metal surfaces due to the contact between the metal surface and bacterial cells, as well as extracellular polymeric substances. The roughness of the metal surface and the build-up of charge are further factors that might impact the development of biofilms. Once they are formed, bacterial metabolites and EPS that are produced across the surface of the metal may increase the rate of corrosion in many different metals. It is also believed that electron transport channels are to blame for MIC [66].



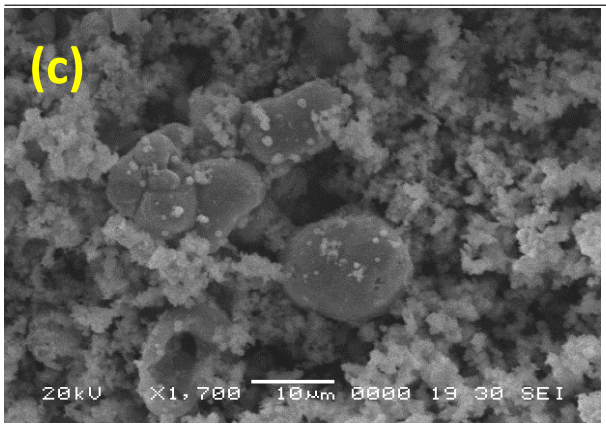


Fig. 7: Show SEM examinations for the biofilm developed on carbon steel surface in a modified CFA medium for 35 days at different magnifications (a) 200 X, (b) 500 X, and (c) 700 X.

The diffraction scan that was acquired and is shown in Fig. 8 and the products that were identified and are represented in Table 4 illustrates the XRD patterns of the corrosion products that were formed on the carbon steel when the bacterial strains were present. After the biocorrosion tests, XRD provided conclusive evidence for the existence of ferric oxide as a corrosion product. Indicating that IOB can oxidize inorganic compounds that are present on the surface of iron coupons and use them as a source of inorganic material for their development, the existence of these iron particles in the corrosion products of bacterial systems shows that these particles may be found [67].

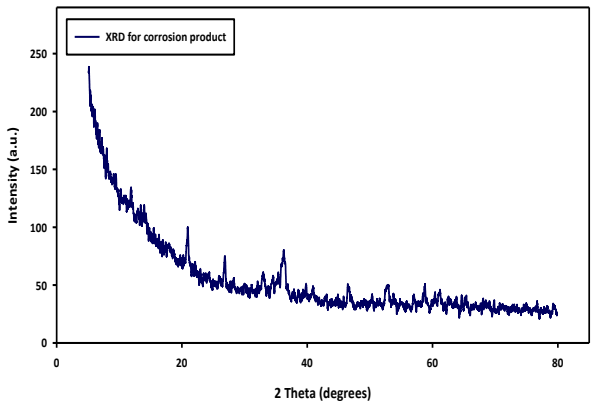


Fig. 8: Shows XRD scan for the corrosion products of the coupons

Table 4: Shows the corrosion products compounds of iron coupon

Compound Name	Chemical Formula
Iron ringwoodite, syn	$Fe_2(SiO_4)$
Lepidocrocite	Fe^{+3} O(OH)
Rostite	(Al, Fe) (SO_4) (F, OH) 5 H_2O
Goethite	Fe^{+3} O(OH)
Wuestite, syn	Fe.942 O
Siderite	$FeCO_3$

3.6. Gas chromatographic analysis of the garlic extract

An examination of sulfur compounds using GC and GC-MS. The distinctive aroma of allium crops, most notably garlic, allows for easy identification of these edibles. Garlic may be differentiated from other allium plants on a biochemical level in several different ways. The aroma of garlic comes from volatile sulfur compounds, such as diallyl disulfide, which give garlic its characteristic flavor.

The examination of these volatile chemicals may benefit from the use of gas chromatography. The mass spectrometer is one of the universal detectors that can be found in GC, and it can identify many volatile substances. On the other hand, the flame photometric detector exhibits selectivity for sulfur or phosphorous atoms, and as a result, it may be used in the process of selectively detecting sulfur compounds. When garlic cloves are crushed, the amino acid alliin transforms into allicin by undergoing a chemical reaction with the enzyme alliinase.

Alliin is the primary sulfur molecule found in fresh garlic that has not been damaged. Because of its volatility and reactivity, allicin cannot be identified in many biological samples. On the other hand, when samples of freshly produced garlic are inserted into the GC chromatogram, peaks that correspond to volatile sulfur compounds may be recognized. These peaks are visible. To form vinyl dithiins, which are identified as the primary peaks of garlic, allicin must first be destroyed either at the injection port of the GC or inside the organic solvent.

This GC approach seems to help detect garlic; however, elephant garlic also displays peaks for vinyl dithiins in its profile. The GC chromatograms of garlic and elephant garlic were distinct from one another, although this distinction could be explained only by the disparity in the amounts of their constituents. As a result, GC is probably not an appropriate method for differentiating garlic from the other plants in the Allium genus.

3.6.1. Chemical composition of garlic extract

The chemical makeup of garlic extract was determined using GCMS and FTIR analysis; the findings were presented in this paragraph. Table 5 is a listing of the primary chemicals that were identified in garlic extra (Allium sativum) using GC-MS.

The use of gas chromatographic analysis allowed for the verification that garlic extracts do contain organosulfur compounds. The GAE produced mostly diallyl disulfide, which has the chemical

formula C6H10S2. Other sulfur-containing compounds, such as allyl trisulfide, were found in the GAE extracts as well (Table 5 and Fig. 9).

Table 5: Constituents of Allium sativum. Extract and their relative percentages of total chromatogram area and Kovats index

Compounds	Chemical Formula	RT	Area Sum %
1,2-Dithiolane	$C_3H_6S_2$	3.156	0.62
Diallyl sulfide	$C_6H_{10}S$	3.391	0.44
Allyl methyl disulfide	$C_4H_8S_2$	4.661	1.7
1,2-Dithiacyclopentene	$C_6H_{11}ClS$	5.805	1.51
Diallyl disulfide	$C_6H_{10}S_2$	10.217	21.57
Allyl (E)-1-Propenyl disulfide	$C_6H_{10}S_2$	10.623	0.86
Allyl (Z)-1-Propenyl disulfide	$C_6H_{10}S_2$	10.858	3.4
Allyl methyl trisulfide	$C_4H_8S_3$	12.002	6.85
3-Vinyl-1,2-dithi-4-ene	$C_6H_8S_2$	13.513	2.9
4H-1,2,3-Trithiine	$C_3H_4S_3$	13.719	4.85
2-Vinyl-4H-1,3-dithiine	$C_6H_8S_2$	14.343	6.77
Allyl trisulfide	$C_6H_{10}S_3$	16.958	37.35
Allyl propyl trisulfide	$C_6H_{12}S_3$	17.249	0.76
5-Methyl-1,2,3,4-tetrathiane	$C_3H_6S_4$	18.508	1.39
1,2-Dithiole	$C_3H_4S_2$	19.584	1.71
Diallyl tetrasulfide	$C_6H_{10}S_4$	22.96	2.47
Octathiocane	S_8	32.951	3.81
3,11a-Dimethylhexadecahydro-3H-naphtho [2',1':4,5] indeno [1,7a-c] furan-1-one	$C_{16}H_{10}O$	46.226	0.51

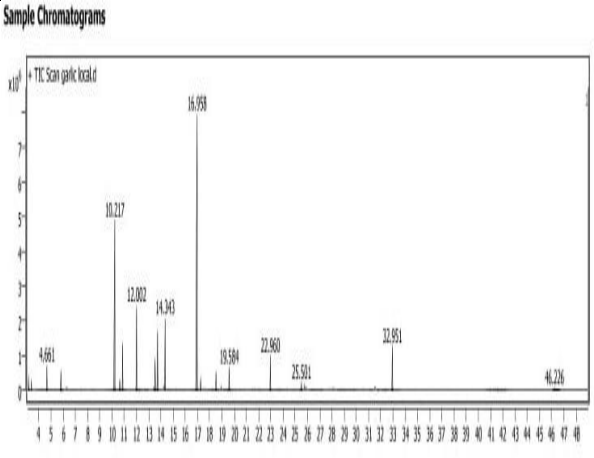


Fig. 9: GCMS spectra of garlic extract

These chemicals have been identified as having been present in GAE in the past [68]. The FT-IR spectra of garlic extract are shown in Fig. 10. It was discovered that the stretching of amides (C=O) is responsible for the –OH stretch at 3383.5 cm^{-1} in the garlic extract, the C-H stretch at 2930.31 cm^{-1} in the same extract, and the band at 1643.05 cm1 in the same extract.

The band that was discovered at 1405.85 cm^{-1} could be because carboxylates have a –O–H bend in their structures. The bands at 1131.05 cm^{-1} and 1024.98 cm1 may be ascribed to SO_2 (sulfones), while the bands at 1131.05 cm^{-1} and 1024.98 cm^{-1} can be attributed to C–N stretching vibrations caused by the presence of amines. The -C–H bond is given a frequency of 931.45 cm^{-1}, the N–H bend of primary amines has a frequency of 816.706 cm^{-1}, and the C–H bend of alkynes has a frequency of 530.328 cm^{-1}.

The sulfide and nitrogen-based compounds that were found in the garlic extract were mostly responsible for endorsing the expected peaks as having occurred.

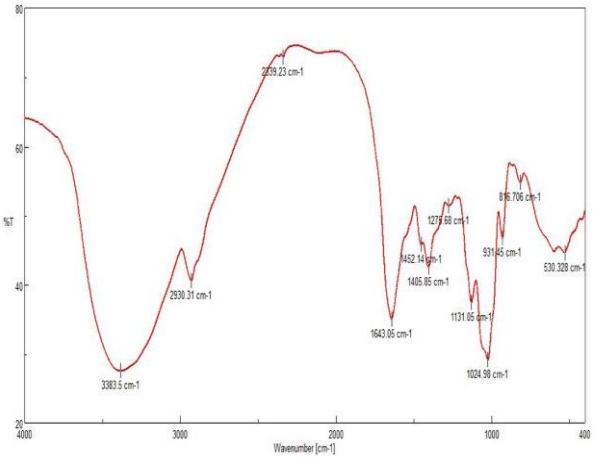


Fig. 10: FTIR spectra of garlic extract

3.7. Efficiency of garlic extract on the isolated bacteria and fungi

Finding the minimum inhibitory concentration (MIC) for garlic extract. In the colorimetric INT- formazon assay, garlic extract demonstrated antibacterial activity that was both efficient and reproducible against all of the bacterial strains that were tested. For all of the tested isolates, minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of garlic extract at concentrations of 20 l per 100 l were adequate. With a mean zone of inhibition of 44.2 and 34.2, respectively, L. Ochracea and T. Ferrooxidans were the bacteria that were impacted to the greatest

extent. Additionally, G. Ferruginea, C. Aggregatum, and A. Castelli were impacted, and the findings of the investigation are shown in Table 6.

Table 6: Antimicrobial efficiency of garlic juice

Isolated bacteria	Clear zone (mm)	Isolated fungi	Clear zone (mm)
G. Ferruginea	29±1	Aspergillus Flavus	26±1
L. Ochracea	44±2	Aspergillus Fumigatus	33±0.5
T. Ferrooxidans	34±2	Aspergillus Niger	42±1
C. Aggregatum	21±1	Aspergillus Sydowii	36±2
A. Castelli	18±0.5	Rhizopus Sp.	27±0.5
		Ulocladium Botrytis	48±1

This is because the biologically active element of Allium sativum, known as allicin, is responsible for its activity. Allicin works largely by inhibiting the sulfhydryl enzymes that are required for bacterial metabolism and by interfering with RNA synthesis. It would seem that thiol-disulfide exchange interactions between sulfur compounds and free thiol groups of bacterial enzymes, such as alcohol dehydrogenase, thioredoxin reductase, trypsin, and other proteases, RNA and DNA polymerases, were responsible for the antibacterial activity of garlic [69].

The FTIR and GC analyses helped to identify the bioactive functional groups present in the garlic extract that we had. This disturbance may affect the critical metabolism of the cell, and as a result, the virulence and development of the bacteria [70]. The production of the enzyme alliinase (lyase) is facilitated by the crushing and mechanical breaking of garlic bulbs. Alliin is broken down into allicin, pyruvic acid, and ammonia when subjected to its effect. Allicin is a volatile byproduct that contributes to the pungent smell of garlic. Both Gram-positive and Gram-negative bacteria are susceptible to the bactericidal effects of the compound allicin ($C_6H_{10}OS_2$).

Allicin is very unstable, and it may break down into several other compounds, including ajoene, vinyldithiin, allixin, DAS (diallyl sulfide), DADS (diallyl disulfide), and DAT (diallyl trisulfide). The antimutagenic capabilities of these compounds, except for vinyldithiin, have been shown [71]. Antibacterial and antiplatelet aggregation are two of the many functions that vinyldithiin has.

Additionally, the antibacterial molecule known as ajoene ($C_9H_{14}OS_3$), which is found in garlic, can block the genes involved in pathogenicity that are regulated by quorum sensing [72]. It is interesting to note that, in contrast to other species, Ulocladium botrytis and Aspergillus niger were shown to have a high susceptibility to garlic juice see Fig. 11. The mean inhibition zone was 48-1, whereas the mean inhibition zone was 42-1. The Aspergillus Sydowii and the Aspergillus fumigatus strains were next in line. Last but not least, Aspergillus Flavus and Rhizopus sp. were the fungi that were damaged the least. It was shown that allicin in its pure form derived from freshly crushed garlic exhibited several kinds of fungus, and the results of much prior research corroborate the findings of the present study [73].

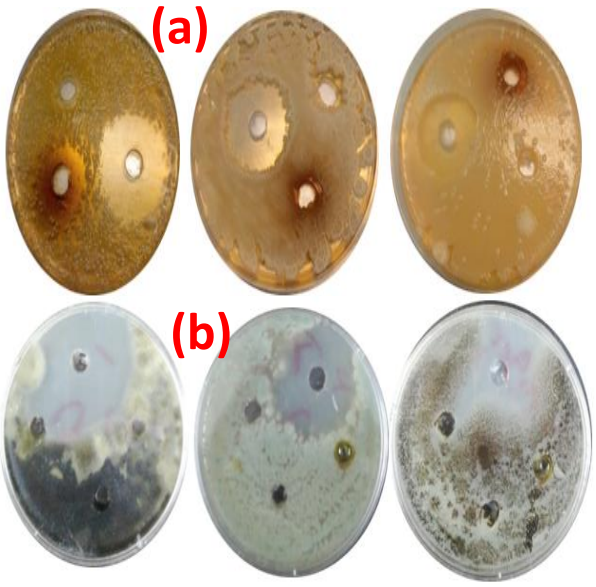


Fig. 11: Representative the antimicrobial efficiency of garlic extracts on (a) isolated bacteria and (b) isolated fungi

3.8. The mechanism of garlic extracts as a biocide

According to the findings, garlic extract has antibacterial properties and was extremely effective against bacterial strains. This influenced the activity of biofilms and led to a reduction in the rates of biocorrosion that occurred under these circumstances. This inhibition was generated by the phytochemical components of the GAE that had been adsorbed, and it may have been able to prevent corrosion on the surface of the carbon steel by controlling biofilm growth [74]. Due to the presence of bactericidal components, particularly diallyl disulfide and trisulfide, garlic extract is very effective in killing bacteria, as seen in Table 6.

On the other hand, the suppression of fungus that was shown in this research might be ascribed to

allicin or ajoene, both of which limit the activity of several enzymes associated with fungi. Both bacterial strains were very sensitive to the garlic extract, as shown by the results of the biofilm inhibition test. In addition to that, it was crystal obvious from the XRD analysis that was carried out with the different bacterial strains and the garlic extract systems. All the features indicate the fact that the bacterial activity was different when the garlic extract was present as opposed to when it was missing, and they all point in the same direction.

It was shown that an extract of garlic might protect metals against corrosion by a process called adsorption, in which the organic components of the extract bind to the surface of the metal [75]. It is common knowledge that biofilm can form on the surface of metal due to the interaction of electrostatic forces, contact between the metal surface and bacterial cells, and the presence of extracellular polymeric molecules.

These three factors combine to create the conditions necessary for biofilm formation. The roughness of the surface of the metal and the build-up of charges are both factors that might influence the creation of biofilm. When biofilms are produced, the bacterial metabolites and extracellular biopolymer that are created over the surface of the metal might potentially induce an increase in the rate of corrosion in several different metals.

According to the results of this investigation, garlic extract has antibacterial properties in addition to its ability to reduce corrosion [76]. These characteristics changed the activity of the biofilm, which in turn led to reduced rates of biocorrosion in an environment that was corrosive. This inhibition was caused by the phytochemical components of the garlic extract that were absorbed, and it is possible that these components might have prevented the formation of biofilm and protected the metal surface from corrosion. This inhibition was caused by the phytochemical components of the garlic extract that were absorbed [77].

3.9. The application of garlic extracts as a biocide on the studied object

According to the results of the examination, the knife is in excellent metallic condition; nevertheless, it is covered with thick layers of corrosion products; as a result, these products need to be removed before the biocide can be applied. After using mechanical cleaning methods to remove corrosion products from the artifact, garlic extract was used as a biocide to destroy any leftover bacteria. This was done to ensure the integrity of the artifact [78].

We went with a chemical treatment first, and then after that, we gave it a careful going through with some skilled mechanical cleaning. Because of this, we were able to disclose the natural topography of the surface. The following pursuits were included as components of the treatment program:

Dissolving the corrosion layers and the calcareous deposits on the blade of the knife by completely submerging it in a solution of one percent sodium hydroxide was done many times.

This was reinforced with a delicate mechanical cleaning done with a silk brush on occasion. After completing this process, the knife was then submerged in water and washed with a toothbrush to remove any residue that may have been lodged in the crevices. The calcareous deposits and the ferric oxide were successfully removed thanks to the implementation of this procedure [79].

Multiple washes in tubs of hot deionized water, with varying degrees of heating and cooling, to ensure that all chemical residues are eliminated and that capillaries are flushed out. After being dried in multiple baths of ether and ethanol, the item is next dried in hot sawdust, and finally, it is dried by being brushed dry with a clean, soft towel. On the surface of the knife, garlic extract was painted with a brush to separate it. Using a brush and some paraloid B-72 that had been dissolved in a third of acetone, the knife was finally separated (Fig. 12)

Fig. 12: Shows the knife after treatment and conservation

Conclusions

The analysis of garlic extract (GAE) as a potential biocide, in addition to the study of the biological deterioration of an archaeological knife that had been recovered from a burial site. According to the findings of this research project, garlic extract has the potential to perform as an effective, eco-friendly, and natural green corrosion inhibitor with biocidal effects when it is applied to carbon steel that has been enriched with bacteria that cause corrosion.

This finding indicates that garlic extract has the potential to perform in this capacity. This is the inference that can be made from the information

provided in the results of the research. Due to the presence of sulfur-rich components, garlic extract has a significant impact on the process of reducing corrosion. This influence is due to the presence of garlic extract. Garlic extract was used, which is why this effect was produced. Based on these results, garlic extract is suggested here as a corrosion inhibitor that is beneficial to the environment and has biocidal characteristics for the treatment of MIC.

It has been hypothesized that the extract of garlic may be exploited as a possible natural biocide that could be used in place of commercial biocides that are used more frequently but pose a greater risk. This has led to the development of a potential natural biocide that has been given the name "garlic extract". More studies on the antibacterial benefits of garlic, particularly concerning Gram-positive and Gram-negative bacteria, would be beneficial.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Not available

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